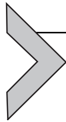

CHAPTER TWENTY SEVEN

# Transformation of *E. coli* Via Electroporation

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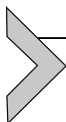
## Abstract

To create electro-competent *E. coli* and transform them with a plasmid of choice via electroporation.



## 1. THEORY

Electroporation of *E. coli* is a popular alternative to traditional heat-shock transformation of chemically competent cells. A high-voltage current

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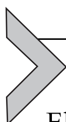
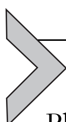
is applied to the cells, which temporarily permeabilizes the plasma membrane and allows DNA or other small molecules to enter. The main advantages of electroporation over heat-shock transformation are the higher efficiency in the uptake of plasmid DNA and a faster, less involved production of competent cells.



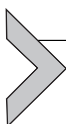
## 2. EQUIPMENT

Electroporator for bacteria (e.g., Gen Pulser Xcell Electroporation System, Bio-Rad)
Refrigerated centrifuge
Refrigerated microcentrifuge
Shaking incubator (37 °C)
Incubator (37 °C)
15-ml sterile polypropylene snap-cap tubes
Electroporation cuvette, 0.1 cm gap
15-ml glass centrifuge tube
Rubber adaptors (to fit glass centrifuge tube into floor centrifuge rotor)
Pipettes
Micropipettors
Micropipettor tips
1.5-ml microcentrifuge tubes
Kimwipes



## 3. MATERIALS

Plasmid DNA (to be transformed)
LB agar plates (selective plates containing appropriate antibiotic)
Lysogeny broth (LB)
Bacto agar
Sterile dd$H_2O$
Glycerol (optional)



## 4. PROTOCOL

### 4.1. Preparation

Inoculate 5 ml of LB with *E. coli*. Grow overnight at 37 °C with shaking.

Chill the 15-ml glass centrifuge tube, a 1.5-ml microcentrifuge tube, the electroporation cuvette, and the $ddH_2O$ on ice. Run the floor centrifuge for a few minutes to cool the chamber to 4 °C.

## 4.2. Duration

| Preparation | 15 min + overnight |
|---|---|
| Protocol | About 2 h |

## 4.3. Tip

*For most simple plasmid transformations, it is not necessary to harvest bacteria at early to mid log phase. However, if the transformation efficiency is low, dilute 1 ml of the overnight culture in 20 ml of LB and grow at 37 °C with shaking until the* $OD_{600}$ *measures between 0.4 and 0.6 (1–1.5 h). Use 10 ml to make 50 μl of competent cells as described subsequently.*

## 4.4. Tip

*For all steps, keep reagents, tubes, and bacteria on ice at all times to ensure the production of high-quality electro-competent cells. Keeping the bacteria at a low temperature during the electric pulse also helps prevent electrically induced heating and thus increases cell survival.*

See Fig. 27.1 for the flowchart of the complete protocol.

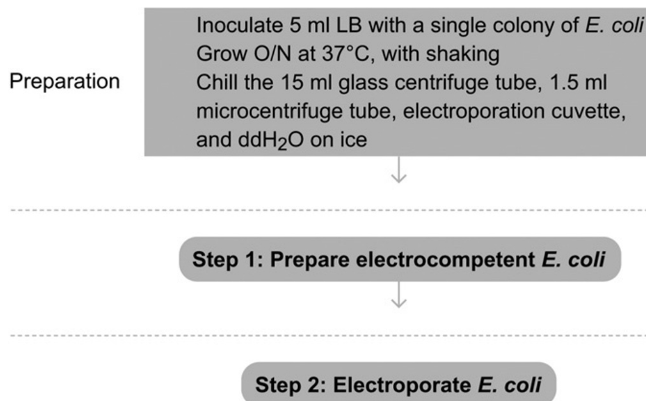
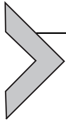



**Figure 27.1** Flowchart of the complete protocol, including preparation.



# 5. STEP 1 CREATE ELECTRO-COMPETENT *E. COLI*

## 5.1. Overview

*E. coli* are washed several times with ice-cold water to prepare them for the uptake of plasmid DNA during electroporation.

## 5.2. Duration

20–30 min

**1.1** Pour the bacterial culture into the prechilled 15-ml glass centrifuge tube on ice.
**1.2** Centrifuge at 7000 rpm at 4 °C, for 5 min.
**1.3** Discard the supernatant.
**1.4** Resuspend the pellet in 1 ml of sterile, ice-cold dd$H_2O$ and transfer into a chilled 1.5-ml microcentrifuge tube.
**1.5** Spin in a microcentrifuge at 7000 rpm at 4 °C, for 5 min.
**1.6** Discard the supernatant.
**1.7** Resuspend the pellet in 1 ml of ice-cold dd$H_2O$.
**1.8** Repeat steps 1.5–1.7 two more times for a total of three washes.
**1.9** Spin a final time at 7000 rpm at 4 °C, for 5 min, discard the supernatant, resuspend the pellet in 50 μl of ice-cold dd$H_2O$, and put the cells on ice.

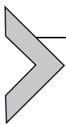
## 5.3. Tip

*If you want to store the electro-competent cells at −80 °C for later use, substitute 10% glycerol for dd$H_2O$ in Step 1.4 and in all subsequent steps.*

## 5.4. Tip

*This protocol can be scaled up to make larger quantities of competent cells.*

See Fig. 27.2 for the flowchart of Step 1.



# 6. STEP 2 ELECTROPORATION OF *E. COLI*

## 6.1. Overview

Plasmid DNA is added to electro-competent *E. coli*. An electric pulse mediates the uptake of the DNA by the bacteria.

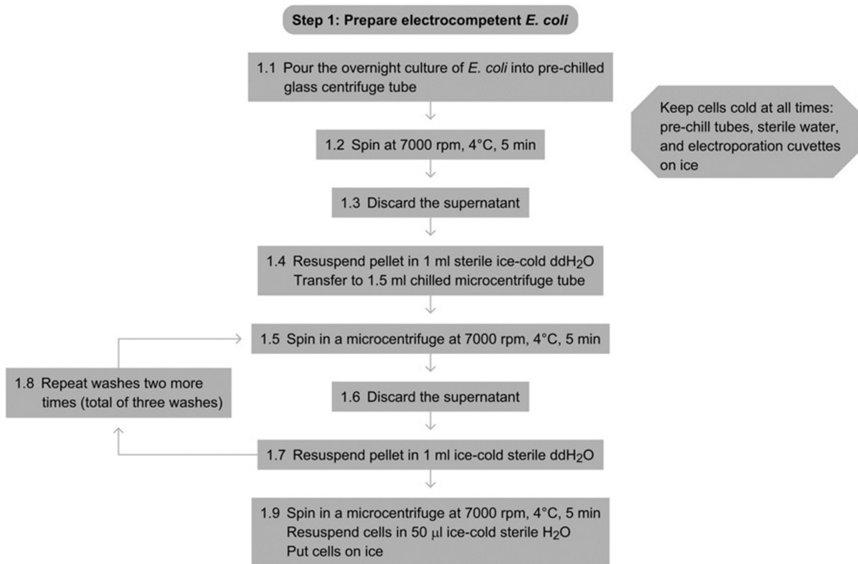



**Figure 27.2** Flowchart of Step 1.

## 6.2. Duration

1 h and 10 min

**2.1** Add 50–100 ng of supercoiled plasmid to the electro-competent *E. coli* and mix gently (do not pipette up and down).

**2.2** Transfer the bacteria to a chilled electroporation cuvette. Be careful to pipette straight in between the metal plates and avoid introducing any bubbles.

**2.3** Cap the cuvette and tap it lightly on the bench to settle the bacteria/ DNA mix.

**2.4** Put the cuvette back on ice and carry it to the electroporator.

**2.5** Turn on the electroporator and set it to 1.8 kV, 25 μF, 200 Ω. This is a standard setting for most *E. coli* strains. Other bacterial strains may require an adjustment of the electroporation conditions.

**2.6** Wipe the cuvette briefly with a Kimwipe to remove any residual water or ice, and then place it in the electroporation chamber.

**2.7** Push the pulse button. The time constant displayed should be around 4 ms.

**2.8** *Immediately* after the pulse has been delivered, add 1 ml of LB (or other growth medium, e.g., SOC) to the cuvette and pipette quickly but gently up and down. Be aware that the transformation efficiency

decreases proportionally to the lag time between the electric pulse and the addition of media.

**2.9** Transfer the mixture to a fresh 1.5-ml microcentrifuge tube.

**2.10** Incubate at 37 °C for 1 h with shaking.

**2.11** Evenly spread 100 μl of your transformation onto a selective plate. Electroporation is highly efficient and often yields a very large number of colonies. To ensure that individual, medium-sized colonies can be picked the next day, mix 10 μl of the transformation with 90 μl of LB and spread this 1:10 dilution evenly onto another

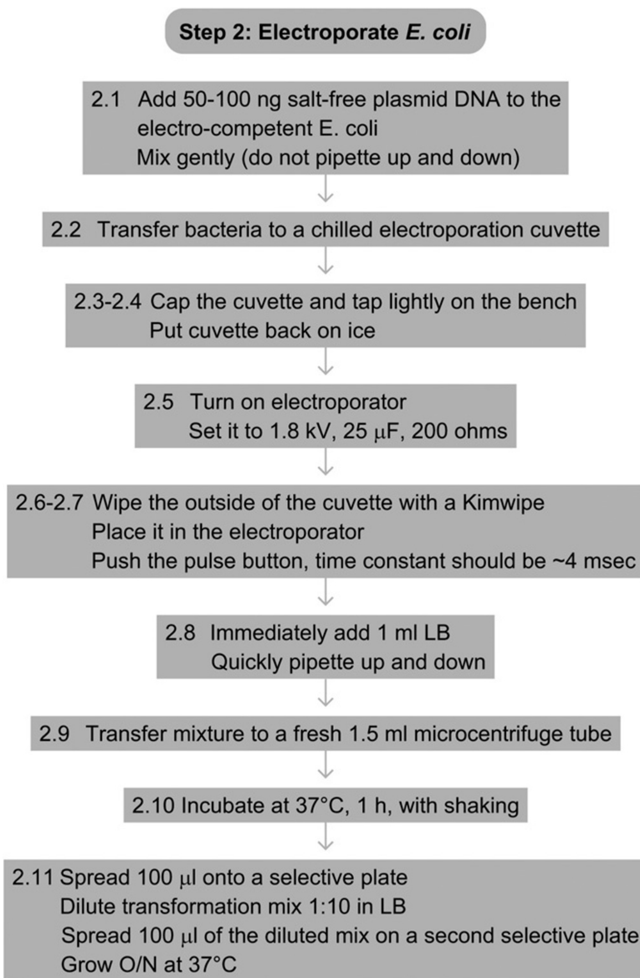



**Figure 27.3** Flowchart of Step 2.

selective plate. Incubate both plates upside down overnight at 37 °C.

## 6.3. Tip

*Using low amounts of well-purified DNA is key to a successful electroporation. Even small amounts of residual salt from DNA preparations may interfere with the electric current and cause arcing, resulting in excessive cell death.*

## 6.4. Tip

*Warm up the selective plates at 37 °C while the bacteria are recovering. Cold plates lead to lower transformation efficiency.*

See Fig. 27.3 for the flowchart of Step 2.

# REFERENCES

### Related Literature

"Gene Pulser Xcell Electroporation System" Instruction Manual, BioRad